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<b>(21) International Application Number:</b> PCT/GB98/02584 <b>(22) International Filing Date:</b> 2 September 1998 (02.09.98)  <b>(30) Priority Data:</b> 9718487.3                      2 September 1997 (02.09.97)                      GB  <b>(71) Applicant (for all designated States except US):</b> UNIVERSITY OF SHEFFIELD [GB/GB]; Western Bank, Sheffield S10 2TN (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> HIGENBOTTAM, Timothy [GB/GB]; University of Sheffield, Section of Respiratory Medicine, floor F, Beech Hill Road, Sheffield S10 2RX (GB). McCORMACK, Keith [GB/GB]; University of Sheffield, Section of Respiratory Medicine, floor F, Beech Hill Road, Sheffield S10 2RX (GB). SMITH, Adrian [GB/GB]; University of Sheffield, Section of Respiratory Medicine, floor F, Beech Hill Road, Sheffield S10 2RX (GB).  <b>(74) Agent:</b> MARKGRAAF PATENTS LIMITED; The Crescent, 54 Blossom Street, York YO24 1AP (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> ANTISENSE TREATMENT OF PULMONARY HYPERTENSION		
<b>(57) Abstract</b> <p>The invention herein described relates to a method to treat pulmonary hypertension by antisense therapy using ET-1 derived antisense molecules delivered to the lungs as a pulse/spike in an inhaler.</p>		

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## ANTISENSE TREATMENT OF PULMONARY HYPERTENSION

The invention relates to a method and means to treat, but not exclusively, pulmonary hypertension by functionally suppressing the product of the ET-1 gene which encodes a vasoconstrictor peptide the expression of which is correlated mainly with this disorder.

Pulmonary Hypertension (PH) is a disorder in which the blood pressure in the pulmonary (lung) arteries is abnormally high in the absence of other diseases of the heart or lungs. PH is considered to be present when the mean pulmonary arterial pressure is greater than 25mmHg at rest or 30mmHg during activity. The normal mean pulmonary - artery pressure is approximately 14mmHg at rest. PH is classified according to whether the disorder is primary or secondary PH.

Primary PH (PPH) is a relatively rare disorder affecting mainly women between the ages of 21 and 40 although men and children can suffer from the disease. The incidence annual rate is as high as 2 per million of the UK population. In the USA approximately 300 new cases of PPH are diagnosed each year. The exact cause of PPH is unknown, this is mainly due to the low incidence of PPH and the lack of an effective animal model in which to study the disease. However PPH is associated with cirrhosis, AIDS and can be familial.

Secondary Pulmonary Hypertension (SPH) is present where there is an identifiable cause resulting in an increase in pulmonary arterial pressure. These include left ventricular failure, chronic hypoxia, lung disease or alveolar hypoventilation and a number of systemic diseases such as lupus

erythromatosis, systemic sclerosis, SPH may also result from chronic thrombo-anabolic disease.

For both PPH and SPH the symptoms are similar. These include breathlessness, syncope (faints), angina (chest pain), headaches, fatigue, blurred vision, insomnia, and intestinal complaints. SPH is much more common and is a major killer in the Western world.

There are currently a number of drug treatments that can help to ameliorate pulmonary hypertension. The successful clinical treatments include the continuous intravenous administration of prostacyclin ( $\text{PGI}_2$ ) and inhaled nitric oxide (NO), for severe disease; for milder degrees of illness, anticoagulants and calcium channel blockers can be used to lessen symptoms and improve survival.

In pulmonary hypertension, sufferers respond differently to different drugs therefore no single drug regime can be used to blanket treat sufferers. Moreover dosages have to be empirically decided due to the potential for adverse side effects. For example, systemic low blood pressure, nausea, angina, headaches and flushing. Therefore alternative strategies are required to provide therapeutic treatment of pulmonary hypertension.

The product of the gene endothelin-1 (ET-1) has been shown to act as a potent vasoconstrictor and is involved in the remodelling of blood vessels of the lung to cause narrowing and obstruction. The product of the ET-1 gene is found in endothelial cells that line arteries and has been shown to be elevated in expression in individuals and in animal models that show pulmonary hypertension. Moreover, current successful treatments of clinical and experimental hypertension using  $\text{PGI}_2$ , and NO have been shown to

inhibit the expression of ET-1. This correlative data is strongly suggestive that ET-1 is an important component in mediating pulmonary hypertension.

Indeed, the drug BQ-123 has been shown to antagonise the activity of ET-1 receptors and relieve pulmonary hypertension resulting from congestive heart failure. In rat models, mRNA to ET-1 was shown to decrease as a  
5 consequence of long term BQ-123 treatment.

The problems of drug side effects, amongst other things, has lead to alternative therapeutic strategies to be devised. A considerable effort is being made toward developing gene therapy approaches to control disease  
10 pathogenesis and it is likely that in subsequent years this will provide a serious alternative to the use of pharmaceuticals.

A recent strategy to interfere with the expression of a gene is that of antisense technology. In brief this strategy involves the use of a DNA or RNA molecule that is complementary to a region of a selected gene and is  
15 able to hybridise (bind) under physiological conditions to the targeted nucleic acid to prevent either transcription of the gene or translation of the mRNA encoded by the gene. The antisense molecule is often a short oligodeoxynucleotide (ODN). However the molecule may be an oligodeoxyribonucleotide, or a modified oligodeoxynucleotide, or a  
20 modified oligodeoxyribonucleotide; each of which are able to hybridised to a selected part of a gene, or mRNA, under physiological conditions. The modifications to oligodeoxynucleotides will be apparent to one skilled in the art. The exact region of the nucleotide sequence of the gene to which the antisense molecule is designed can be empirically determined. However it is  
25 common practice to design oligodeoxynucleotides to the 5' region of the gene (to interfere with transcription initiation) or the mRNA (to interfere

with translation). As stated before, the region of the gene to which the antisense molecule is directed is determined by the efficiency with which the antisense molecule suppresses the gene of interest. Contrary to the above this may be the 3' region and is determined experimentally. The length of the ODN also has to be determined experimentally. Typically ODNs are 20-30 nucleotides in length but may be much longer.

Presently, a number of delivery methods are being explored for their effectiveness at delivering gene therapy to a specific tissue. Viral vectors and liposomes have been used with some success. Recently, cationic lipids chelated to a therapeutic DNA molecule have been shown to deliver the DNA molecule to the lungs (1). Cationic lipids are positively charged lipids which when combined with negatively charged DNA form a tight complex. Moreover, the lungs are coated in a surfactant, a major component of which is dipalmitoylphosphatidylcholine, which is cationic at physiological pH and which is internalised by endothelial cells that line the arterioles of the lungs.

In our co-pending application (GB9805185.7 ref. 2) the delivery of an aerosol containing a vasodilator by a specialised inhaler is described. As an alternative to the continuous perfusion of the lungs using a nebuliser, the therapeutic composition, ie aerosols, is delivered intermittently and in short pulses using the so called "spike principle". By using this methodology the medicament is driven deep into the lung tissue only during inhalation. There are many lung diseases and conditions suffered by humans for which the preferred form of treatment involves delivering a medicament of the appropriate sort down a patient's airways into the lungs, where the medicament can act upon, and perhaps be absorbed into, the tissues of the lungs. The most effective treatments for these disorders involve the inhalation of some suitable chemical agent in inhaled air. The co-pending

application describes the administration of nitric oxide, a known vasodilator, in the form of a pulse or "spike". In the treatment of the constriction of the small pulmonary arteries, the very short pulse of nitric oxide is provided at the start of the inhalation, such that the resultant bolus of nitric oxide mixture  
5 inhaled by the patient has a nitric oxide concentration high enough to have the desired therapeutic effect, even if admixed with some additional air, but is of such short duration (both in time and as a result, in physical amount) that, pushed by the following much larger volume of plain, and therefore nitric oxide - free, air/oxygen, it reaches deeper into the lungs, where it both  
10 acts on the small pulmonary arteries and is taken up into the capillaries,

The invention therein described is a small (pocket-sized) hand held treatment apparatus that utilises the "spike" principle. The invention comprises a conventional nebuliser (having a reservoir in which is stored the medicament to be administered) driven by some suitable pressurised gas from a valved  
15 cylinder so as to deliver a medicament into a tube through which the patient is breathing (by mouth) normal air, the gas cylinder valve being controlled by a suitably programmed computing device that is fed data describing the pressure within the breathing tube and so is able to open and close the valve at, and for, a time such as to drive the nebuliser to deliver (to the tube and  
20 thence to the patient's lungs) a required pulse of medicament at any selected point within the patient's respiratory cycle. The idea of this is simply to ensure that a high concentration of the medicament reaches and affects the target area, and the target area alone, rather than having the whole of the lungs subjected to it.

25 The apparatus of the invention incorporates a nebuliser in the reservoir of which is storable the medicament to be administered. The reservoir has a fine orifice through which its contents may exit and across which is blown a

stream of gas along a pipe way. As the gas flows past the orifice it causes an external pressure drop (relative to the reservoir's internal pressure) resulting in some of the reservoir's contents being sucked out into the gas stream creating an aerosol-like cloud of fine drops or particles that is swept along the pipe way by the driving gas. The driving gas is contained within a valved cylinder, the operation of the valve being controlled by a computing means in response to the input from a pressure sensor. The pressure sensor is located within the breathing tube and provides the computing means with data about the air pressure there within, and thus about the patient's respiratory cycle.

The invention therefore comprises a nebuliser with a reservoir containing a therapeutic composition which has contact with a gaseous supply through which the therapeutic composition is aerosolised. The delivery of the composition via the breathing tube is regulated by a valved cylinder which operates according to pressure data received from a pressure sensing means located in the breathing tube to determine the stage of the patient's respiratory cycle. The whole system is computer controlled to ensure that the pulse of therapeutic composition is delivered at the appropriate stage of the patient's respiratory cycle.

We have used an inhaler employing the above 'spike' methodology and in one example the specialised inhaler described in GB9805185.7 to create an aerosol of ET-1 antisense molecules to perfuse lung tissue to relieve pulmonary hypertension. The designed antisense ET-1 molecules will target either the ET-1 gene and/or the ET-1 mRNA to suppress the ET-1 gene product from the endothelial cells that line the lung arterioles and thereby relieve pulmonary hypertension.



It is therefore an object of the invention to design antisense ODNs to ET-1 for use in the alleviation of pulmonary hypertension.

It is yet a further object of the invention to deliver antisense ODNs to the lungs to alleviate pulmonary hypertension.

- 5 According to a first aspect of the invention there is provided a method to treat pulmonary hypertension comprising administering a therapeutic composition to an individual, wherein said composition comprises at least one aerosolised antisense ET-1 molecule, delivered to the lungs by an inhaler during inhalation by a patient.

10

Ideally said antisense material is of mammalian origin, and most ideally of human origin.

- It will be apparent from an analysis of the prior art that the biological effects of ET-1 are mediated through the receptors ET-A and ET-B. Therefore  
15 although embodiments of the invention will be described that target ET-1 with antisense ET-1 molecules it will be apparent that targeting ET-A and/or ET-B may achieve the desired result of suppressing ET-1 activity although not directly. Therefore the following description assumes that one skilled in the art would appreciate that the antisense strategy could be applied to  
20 suppress ET-A and/or ET-B activity.

According to a further aspect of the invention there is provided a method to treat pulmonary hypertension comprising delivering aerosolised antisense ET-1, or ET-A or ET-B molecules to the lungs by an inhaler as a pulse/spike during inhalation.

According to yet a further aspect of the method there is provided a therapeutic composition for treating pulmonary hypertension comprising; the non-coding strand of the ET-1 gene, or fragment thereof, as represented in Figure 1.

5

It will be apparent from Figure 1 that the sequence presented is the sense strand of the ET-1 gene. Antisense molecules would be designed to represent sequence complementary to this sequence and can be easily deduced from the sense sequence.

10

In a preferred method of the invention said antisense ET-1 molecule is a oligodeoxynucleotide, a modified oligodeoxynucleotide, an oligodeoxyribonucleotide or a modified oligodeoxyribonucleotide represented by the DNA sequence shown in Figure 1, that is the non-coding strand of ET-1.

15

Reference herein to modifications is intended to include without limitation modifications apparent to those skilled in the art that improve stability (both as a stored composition and during *in vivo* use; improve permeability across biomembranes or facilitate aerosolisation).

20

In a preferred method of the invention the antisense molecule is between 10-100 nucleotides in length.

In yet a further preferred method of the invention said antisense molecule is greater than 100 nucleotides in length.

In yet still a further preferred method of the invention said oligonucleotide has a G + C content of at least 50% and more preferably still a G + C content of between 50% and 60%.

- 5 More preferably still said oligonucleotides do not contain a consecutive run of more than four bases of any one kind.

In yet still a further preferred embodiment of the invention said oligonucleotide has a predicted melting temperature of at least 55<sup>0</sup>, and more  
10 preferably still, a melting temperature of between 55<sup>0</sup> – 80<sup>0</sup>.

In a preferred embodiment of the invention there is provided at least one human ET-1 antisense oligonucleotides as represented in Table 1.

15

Table 1

HsET117	CAG CCC AAG TGC CCT TTA AC
HsET132	CTC AAA GCG ATC CTT CAG CC
20 HsET318	AGC TCA GCG CCT AAG ACT GC
HsET438	TGG CAG AAG TAG ACA CAC TC
25 HsET779	TGG TCT CCG ACC TGG TTT GT
HsET868	ATG TGC TCG GTT GTG GGT CA

In yet a further preferred embodiment of the invention there is provided at  
30 least one rat ET-1 antisense oligonucleotides as represented in Table 2.

Table 2

RnET198	ACA GCA GAG AGA AGA TCA CG
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RnET294    TGC ACT TCC TTC TCA GCT CG  
RnET466    GGA TCG CTT AGA CCT AGA AG  
5    RnET718    CTT GAT GCT GTT GCT GAT GG  
RnET795    AGT CAA TGT GCT CGG TTG TG  
10    RnET1081    ACT GTG TCT CTG CTC TCC GA

It will be evident to one skilled in the art, and from the above description, that a reliable animal model to test the efficacy of therapeutic treatments for pulmonary hypertension currently does not exist. It would therefore be desirable to develop an animal model system to test the efficacy of antisense ET-1 molecules. The sequences identified in table 2 represent preferred rat sequences. However it will be apparent to one skilled in the art that oligonucleotides derived from other species may also be useful in practising this method of the invention. Particularly those sequences that have a high degree of sequence homology to those sequences presented in Tables 1 and/or 2.

According to a further aspect of the invention there is provided a method for determining the efficacy of antisense ET-1 molecules comprising exposing a known hypersensitive animal model system to antisense ET-1 for studying molecules and observing the effects of same on pulmonary hypertension.

In a preferred embodiment of the invention said therapeutic treatment is based on antisense therapy. Ideally said antisense therapy is based on the ET-1 sequence and more preferably still those sequences represented in Table 1 and/or Table 2, and/or sequences homologous or analogous thereto,

wherein said homology is at least 50%, ideally 75% and preferably at least 90%.

In yet a further preferred method of the invention there is provided a modified antisense ET-1 molecule as herein described for the treatment of pulmonary hypertension.

It will be apparent to one skilled in the art that a number of potential  
5 modifications can be made to the antisense oligodeoxynucleotide molecules to improve efficacy and/or stability of said molecules. These include modifications to the phosphodiester bonds between nucleotides, (eg the inclusion of peptide bonds to form peptide nucleic acids). Other  
10 modifications may be to the bases and/or sugars by the covalent attachment of chemical groups to specific sites in the sugar and/or base. All of these modifications will not affect the binding properties of the antisense molecule to its target site in ET-1.

According to a further aspect of the method there is provided an antisense molecule adapted to hybridise to the transcriptional promoter region of the  
15 ET-1 gene to inhibit transcription of said gene.

It will be apparent from an analysis of the prior art that the biological effects of ET-1 are mediated through the receptors ET-A and ET-B. Therefore although embodiments of the invention will be described that target ET-1 with antisense ET-1 molecules it will be apparent that targeting ET-A and/or  
20 ET-B may achieve the desired result of suppressing ET-1 activity although not directly. Therefore the following description assumes that one skilled in the art would appreciate that the antisense strategy could be applied to suppress ET-A and/or ET-B activity.

It will be apparent to one skilled in the art that the antisense ET-1 molecule used in this way can be designed to either the sense strand or antisense strand of the ET-1 transcriptional promoter as the target sequence is double stranded genomic DNA.

- 5 According to yet a further aspect of the invention there is provided an antisense molecule adapted to hybridise to splice junctions between exons and introns of prepromessenger RNA encoding the ET-1 protein.

10 It will be apparent to one skilled in the art that the expression of the ET-1 gene can be inhibited by interfering with the splicing of introns from the pre mRNA of ET-1 to prevent the formation of a mature, translatable mRNA.

According to a further aspect of the invention there is provided an inhaler comprising means for administering the aforescribed antisense molecules encoding ET-1 nucleic acid wherein said administration is via a spike or  
15 pulse and preferably only during inhalation by the patient.

According to yet a further aspect or embodiment of the invention said inhaler is disposable and adapted to receive a cartridge containing antisense molecules encoding the ET-1 nucleic acid composition.

- 20 According to yet a further aspect of the invention there is provided a method for administering the composition of the invention comprising;

- i) adding or providing the antisense molecular composition in a suitable diluant, carrier or excipient and in dosage form to, or in a reservoir of, an inhaler;

- ii) aerosolising said composition by a suitable means;
- iii) detecting the air pressure within the inhaler by a suitable sensing device to determine a patient's respiratory cycle; and
- iv) delivering a prescribed dose of the afore described therapeutic composition to the patient's lungs during a selected inhalation phase of the patient's respiratory cycle.

In yet a further aspect of the method there is provided a kit used to treat pulmonary hypertension comprising; a therapeutic antisense composition as hereindescribed; an inhaler, including a nebuliser, a reservoir for containment of said composition, an air pressure sensing device and a valve containing pressurised cylinder means to regulate the dosage of said composition.

The invention hereindescribed therefore provides a means of controlling pulmonary hypertension using aerosolised antisense molecules to the ET-1 gene, and/or mRNA, to inhibit the vasoconstrictor activity of the ET-1 peptide. Moreover, the delivery of the antisense molecule makes use of an inhaler that functions during patient inhalation to drive the therapeutic composition deep into the lungs to deliver the antisense molecule to the endothelial cells that line the arterioles of the lungs and thereby target those blood vessels that result in pulmonary hypertension.

An embodiment of the invention will now be described, by example only, and with reference to the following Figure and methods wherein;

Figure 1 represents the genomic DNA sequence of human ET-1.

### Antisense Design Protocol

#### Table 1

##### 5 Human sequences

HsET117	CAG CCC AAG TGC CCT TTA AC
HsET132	CTC AAA GCG ATC CTT CAG CC
HsET318	AGC TCA GCG CCT AAG ACT GC
10 HsET438	TGG CAG AAG TAG ACA CAC TC
HsET454	CCA AAT GAT GTC CAG GTG GC
HsET779	TGG TCT CCG ACC TGG TTT GT
HsET868	ATG TGC TCG GTT GTG GGT CA

##### 15 Table 2

##### Rat sequences

RnET198	ACA GCA GAG AGA AGA TCA CG
RnET294	TGC ACT TCC TTC TCA GCT CG
RnET466	GGA TCG CTT AGA CCT AGA AG
20 RnET718	CTT GAT GCT GTT GCT GAT GG
RnET795	AGT CAA TGT GCT CGG TTG TG
RnET1081	ACT GTG TCT CTG CTC TCC GA

The above sequences were designed by identifying regions of loose  
25 predicted secondary structure and choosing sequences of 20 base pairs  
meeting the following criteria;

- a) 50-60% G+C content
- b) predicted melting temperature of 55-80 °C



c) <4 runs of bases of any kind

Regions of loose secondary structure were defined as sequences of >8 adjacent bases in the mRNA sequence for which there were no predicted  
5 intra-molecule Watson-Crick base pairing determined using the Wisconsin Genetics Computer Group software package RNAFOLD.

The mRNA sequence was then imported into the 'primer' package on the Sheffield 'biocomp service' and complemented (G for C, T for A etc.). The  
10 regions of loose predicted secondary structure were sought and the oligo criteria were selected. The 3' most bases were aligned with the region of no predicted secondary structure. If the sequence highlighted met all set criteria the sequence and position was recorded. If the sequence failed to reach the criteria the highlighted 20 base sequence was shifted 5' until either a  
15 sequence was identified meeting the criteria or the <5 bases remained within the region of loose predicted secondary structure at which point the region was rejected. When more than one 20 base sequence met the criteria at a particular region of loose predicted secondary structure the sequence with the most central homology to that of the loose predicted secondary structure  
20 was selected.

Current data relating to the efficacy of ET-1 antisense material is *in vitro* cell culture work. Selected antisense molecules are transfected into cells in culture and the level of ET-1 protein determined by western blotting and/or  
25 immunofluorescence using ET-1 antisera. A down regulation of ET-1 protein results when selected antisense molecules are used. Currently we are unable to identify the mechanism by which ET-1 protein is down regulated (ie destabilisation of mRNA, inhibition of translation). Further work will

optimise conditions with respect to providing a suitable composition for use in *in vivo* studies.

The working of the invention is undertaken by using conventional techniques  
5 which will therefore not be described herein in great detail. Suffice to say, that the hereindescribed antisense material is manufactured in conventional manner using laboratory techniques and then suspended in a suitable fluid for the purpose of delivery (3). When thus manufactured the antisense material is suitably contained, ideally in a cartridge, which cartridge is  
10 adapted for use in a conventional inhaler and more preferably the inhaler described herein (2).

In this way, the invention is worked in a conventional fashion.

- 15 1 Delivery of genes using cationic lipid US patent no. 5641662
- 2 Inhalers. UK patent application no. 9805185.7.
- 3 Nyce, J.W. and Metzger, W.J. DNA antisense therapy for asthma in  
20 an animal model. Nature 285: p721-725. 1997.

CLAIMS

1. A method to treat pulmonary hypertension comprising administering a therapeutic composition to an individual, wherein said composition  
5 comprises at least one aerosolised antisense ET-1 molecule, delivered to the lungs by an inhaler during inhalation by a patient.
2. A method according to Claim 1 wherein said composition is delivered to said lungs as a pulse/spike during inhalation.
- 10 3. A therapeutic composition for treating pulmonary hypertension comprising a non-coding strand of the ET-1 gene, or fragment thereof, as represented in Figure 1; and, optionally, in combination with a suitable carrier.
- 15 4. A therapeutic composition according to Claim 3 wherein said non-coding strand of the ET-1 gene is a oligodeoxynucleotide, or a modified oligodeoxynucleotide, or a oligodeoxyribonucleotide, or a modified oligodeoxyribonucleotide.
- 20 5. A therapeutic composition according to Claim 3 or 4 wherein said non-coding strand of the ET-1 gene is between 10-100 nucleotides in length.
6. A therapeutic composition according to Claims 3 or 4 wherein said  
25 non-coding strand of the ET-1 gene is greater than 100 nucleotides in length.
7. A therapeutic composition according to Claims 3-6 wherein said oligonucleotide has a G + C content of at least 50%.

8. A therapeutic composition according to Claims 3-7 wherein said oligonucleotide has a G + C content of between 50% and 60%.
- 5 9. A therapeutic composition according to Claims 3-8 wherein said oligonucleotide does not contain a consecutive run of more than four bases of any single kind.
- 10 10. A therapeutic composition according to Claims 3-9 wherein said oligonucleotide has a predicted melting temperature of at least 55<sup>0</sup> C.
11. A therapeutic composition according to Claims 3-10 wherein said oligonucleotide has a predicted melting temperature of between 55<sup>0</sup> - 80<sup>0</sup>C.
- 15 12. A therapeutic composition according to Claims 3- 11 wherein said antisense ET-1 molecule is of human origin and comprises at least one from;
- i) CAG CCC AAG TGC CCT TTA AC
  - ii) CTC AAA GCG ATC CTT CAG CC
  - 20 iii) AGC TCA GCG CCT AAG ACT GC
  - iv) TGG CAG AAG TAG ACA CAC TC
  - v) TGG TCT CCG ACC TGG TTT GT
  - vi) ATG TGC TCG GTT GTG GGT CA
- or an oligonucleotide sequence homologous therewith.
- 25 13 A therapeutic composition according to Claims 3-12 wherein said antisense ET-1 oligonucleotide is of rat origin and comprises at least one from;
- i) ACA GCA GAG AGA AGA TCA CG

- ii) TGC ACT TCC TTC TCA GCT CG
- iii) GGA TCG CTT AGA CCT AGA AG
- iv) CTT GAT GCT GTT GCT GAT GG
- v) AGT CAA TGT GCT CGG TTG TG
- 5 vi) ACT GTG TCT CTG CTC TCC GA

or an oligonucleotide sequence homologous therewith.

14. A therapeutic composition according to Claims 3- 11 wherein said non- coding strand of the ET-1 gene is adapted to hybridise to the  
10 transcriptional promoter region of the ET-1 gene to inhibit transcription of said gene.

15. A therapeutic composition according to Claims 3- 11 wherein said non-coding strand of the ET-1 gene is adapted to hybridise to splice  
15 junctions between exons and introns of prepromessenger RNA encoding the ET-1 protein.

16. A method for detemining the efficacy of a therapeutic composition in the treatment of pulmonary hypertension comprising administering a  
20 composition according to Claims 3-15 to a selected human or animal and observing the effects of said composition on pulmonary hypertension.

17. An inhaler comprising means for administering the therapeutic composition according to Claims 3-15, wherein said therapeutic  
25 composition is administered via a pulse or spike during use of the inhaler.

18. An inhaler according to Claim 17 wherein the inhaler is disposable.

19. An inhaler according to Claim 17 wherein the inhaler is adapted to receive a cartridge containing antisense molecules according to Claims 3- 15.

20. An inhaler according to Claims 17 -19 wherein said inhaler comprises a removable cartridge wherein said therapeutic composition is stored prior to use.

21 An inhaler according to Claims 17-20 wherein said inhaler comprises a rechargeable cartridge in which said therapeutic composition is stored prior to use.

22. A method for administering a therapeutic composition according to Claims 3-15 comprising;

- i) adding or providing said therapeutic composition in a suitable dilutant, carrier or excipient and in dosage form to, or in a reservoir of, an inhaler;
- ii) aerosolising said composition by suitable means;
- iii) detecting the air pressure within the inhaler by suitable sensing device to determine a patient's respiratory cycle; and
- iv) delivering a prescribed dose of the therapeutic composition to the patient's lungs during a selected inhalation phase of the patient's respiratory cycle.

22. A kit for treating pulmonary hypertension comprising a therapeutic composition according to Claims 3-15, an inhaler according to Claims 17-21, an air pressure sensing device, and a valve containing pressurised cylinder means to regulate the dosage of said composition.

Figure 1

1/7

1 GATATCCTAT TAATACAGAG ATACAGAAAG AAATACATAA AAAATAGTTT TATCAATAC  
 61 TTTCACGACAT TCAAGTGTAG CCTCAAAAGC AAGAATAGGC CAGAGTGGT GGCCTCACGCT  
 121 GTAATCCACA GCACTGTGGG AGGCCAAGGT AAGAGGATTG CTTGAGGCCA GGATTTCAG  
 181 ACCAGCCTAG GCAACATAGT GAGATCCCTA TCTCTACGAA AAAATTTAA AACTTAGCTG  
 241 GGCATGGTGC TTGAGCCTGT TGTCCACAGT ACTCAGGAGG TGAAGTAGGA GTGTCACTTG  
 301 AGCCACAGAG GTTGAGGCTG CAGTGAGCTA TAACCTGCACC ACTGCACCTCC AGCCTTGAG  
 361 ACAGAGTGAG ACCCTGTCCC CAAAAAAATT AAAATTGAGA AAAAAAAGGA GGCAAGACAA  
 421 GCCACAGCAA ACTTCTATT GGGGAAAAAA AAAAATCCTC CTCTTTACAT CTCTCCCTTC  
 481 CTTCCCTTCC CTTCTGAGA GTGACTGTGG CCAAAAGGAG CATTTTCCCC CTGCAGTCTT  
 541 CTGAGGGGTG GGGTGGGGCT ATGAAGCTAT CCTTCATATT CACTCCTTTG TCCAGCTCTT  
 601 TTCACCTCTA GTCTTCTCC CCGCATCTCT GTCTAGCAGT GCCCTTAAGTG GAGGAGGGT  
 661 GGGGCATCA AGCTGTAA AACTGTTTGT TGGGTTCTC CTCTCCTCCCT CATTTCTTGA  
 721 TTTCTGGGAA AATGCTTGGC TGGGAGGCTG CCTGGCGAGT GCCCTAGCTG CCTTCTGTGG  
 781 GCTTGAATGG GGCCTTCCCTC TGCCCCCTACA GGAGGAAAAA GGAGCTGCTG CCAGAGGGAG  
 841 AAATGAGAG ATGACAGAG AAGCAGGTG CCACCCCTCG CCCCTGACAC ACAAGAAAAA  
 901 AGACACGGAA ATTCTCTCTC TCTCTCTCT TCTCTATCT CTCTCTCTCT CCCTCTCTCT  
 961 CTCTCTCTCT CTCTCTCTCA CACACACACA CACACACACA CACACACACA CAGCGCGCG  
 1021 CCGCGCGCGC AGGCACACGT CTTGCAAAAT CAGGATTCAA AGAGACAGGG GCACCATTAT  
 1081 ATTTGGCAGC GTGGGGCTT CCAGTCTGA AATCCTGCAT TCTTCTTAC TATTTACTTT  
 1141 CCCCAGCTC GAGAAAGGCC AGGTGTGGC GGATGGCTGG CCACGTTTG TGTTCGAAT  
 1201 TCATATTCAC GGAATGACAC AGACGGGGCG TGGTAGTGC TGTGGAAGC GCTTGGCAG  
 1261 TTTCAATTTG CCCCACCTCT CCACCTGAAG GCTGGCGTT GCTGGAACCT GCAGGGCAG  
 1321 CCTCAGCAAG GTGGGTGGC GTGAGTGGG GTGGAGAAAG GGACTCCAGC TGAAGTAGAA  
 1381 CCCAGGCTG ACCTGAGAA ATTTGGGAGG GCATGGGGG TGGTTTCCG GTAGGGGCT  
 1441 TGAGGACATG TTGTCCTGA CTGTTGTAG TGTTTGTCA AAGTGGCAA AAGTTAAAA  
 1501 AAAAAAAGT AGGGGAGTC CCTGCCAAGA CATATTTCCC AGGCCACCTT TCTTCCGCGG  
 1561 GAGTGTGGG GGGGAGGCC TGCCTTGAAC CTGTGAATGT GACATCAGCT CTCTCTCTCT  
 1621 CTCCCAAGT CGGCTTTGGA GAGGAGGTC AGGGCACCTT TGCTTGGCAC AGGCAGCTG  
 1681 GCTTCCGGCT CAGTCCGCC TGCTCTCCG GAGCTGTGG CTCCCTGGG CCCGGGGCTA  
 1741 GGTGAGGTA AGCGACAGC GAGGCCAGG CGCGCCGCA GAGCCTGGG GATAGGGTG

Figure 1-2

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1801 GAGGCATCTC TGGGTGTGGG TGTGGGTGTG GGTGTGGAG GGAGATCTT TGCCCTCTCTC  
 1861 TCTCCCATCT CCAACTCTTG CTTCACTGGC TCTTTTAGAG GATGCATGTC ATTATGGACC  
 1921 TGTGCTGCC ACTGTCCCTG TTCCCCACAG TGTGACTTCG AGGAGGTCT GGGATCTGA  
 1981 GTCTGTCCAA ACCCAGGCT TTGCTGTGG GATAAAACAT GTCCCTTTGA TTTTAGAAGG  
 2041 AGGAGGAAA AAGGTTTC CAGCATGTGT GTTGTGCCAG TCTTGGAAT TCATCCGTGC  
 2101 TTGAATTCCA CCTCCATCC CCAGAAAAAC TGGAGTAAAA CAAAAAGAGG AGATGACAA  
 2161 AGTGTGTAAT TGATGGCATC CCTGGGAG AGACTCTAAA TTTATCCCAT AGGTCTTACT  
 2221 GGGCCACTGT GAGCGCTTGT GTGAGAGACA AACAAAAAT CTGGGTGCTC AGTGTCTAA  
 2281 CCTGAAAAAT GGGACTAGCG GAAAAAGCCA ATGTGTCCA TGCACCTTTT GCTTTCTTTA  
 2341 TTAAGGCATG ATGTACCTG TACAGTAACT GCCCTGTGTG TACTTCAGGG GGGATTTCA  
 2401 AGGTTAGATA GACAGGAAAT TGTTTTGAAA ATGTAACAC ATTATTAAT GTGAAGTAT  
 2461 ATCTGATCC TTGTTCGAAT GGCATTTCTT TCTCAGCAC ACCCTTCTTG CATATTTACT  
 2521 TAACCTGTGA CAAGAACACC TTTTGTGCCCT AAATGAAGAC ACCCCCCCAA AAAAAAGAGT  
 2581 CCCAGAAAAAT ATGTCCCTGC TTGTGCGGGA ATAAATAGAA TATTCAGAG TGCATTCCTC  
 2641 CTTCCTATGT TAGGCAACAT TCCCTGAACC TCCTCGGCC CCAAGCCAGG TTGCGTTT  
 2701 TTCTGCCATT TAGAAGGCT TTCCCTTTTG TCCTAGTAAA ACATCAGCC CTGTAGCTCT  
 2761 TCATCTCCCC CTGGTGTCT TCTCCCGCCA TGTCTTAAAG TTGGTGGAC CGACCAATCT  
 2821 TAAGATTAA GTTCTGTGTG AAAAAACACT TTGCTTTTCA ATCAGTTTAT CAGCCTCTC  
 2881 CGCAGGGGAA TGTGGACACA CAAAAAGACT TATCGGGCT TCTCATCAGT GATAGGAAA  
 2941 AGACTGGCAT GTGCCPTAAC GAGCTCTGAT GTTATTTTAA AGCTCCCTTT CTTGCCAATC  
 3001 CCTCACGGAT CTTCTCCGA TAGATGCAAA GAACCTCAGC AAAAAAGACC CGCAGGAAG  
 3061 GCCTTGAAGA GAAAGTACG TTGATCTGCC AAAATAGTCT GACCCCCAGT AGTGGCAGT  
 3121 GACGAGGAG AGCATTTCCCT TGTTTGACTG AGACTAGAAAT CGGAGAGACA TAAAGGAAA  
 3181 ATGAAGCGAG CAACAATTAA AAAAAATTCC CCGCACACAA CAATACAAATC TATTTAAACT  
 3241 GTGGCTCATTA CTTTTCATAC CAATGGTATG ACTTTTTC TGGAGTCCCT TCTTCTGAT  
 3301 CTTGAACCTC GGGCTGGCA GCTTGCAAG GGGAAAGCGA CTCAGGACT GCACGGCAG  
 3361 GTTAGCAAAA GGTCTCTAAT GGGTATTTTC TTTTCTTAG CCTGCCCC GAATTTGTCAG  
 3421 ACGGGGGCG TCTGCTCTG AAGTTAGCAG TGATTTCTT TCGGGCTGG CTTATCTCCG  
 3481 GCTGCACGTT GCCTGTGTGT GACTAATAAC ACAATAACAT TGTCTGGGGC TGAATATAAG  
 3541 TCGGAGCTGT TTACCCCCAC TCTAATAGGG GTTCAATATA AAAAGCCGGC AGAGAGCTGT



Figure 1-3

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3601 CCAAGTCAGA CGCGCTCTG CATCTGCGC AGGCGAACGG GTCCCTGCGC TCCTGCAGTC  
 3661 CCAGCTCTCC ACCGCCGCGT GCGCTGCAG ACGCTCCGCT CGCTGCCCTC TCTCCTGGCA  
 3721 GCGCGTCCCT TTTCTCCCGG TTAAGGCGCA CTTGGCCTGA AGGATCGCTT TGAGATCTGA  
 3781 GGAACCCGCA GCGCTTTGAG GCACCTGAAG CTGTTTTCTT TCGTTTCCCT TTGGGTTGAG  
 3841 TTTGAACGGG AGGTTTTTGA TCCCTTTTTT TCAGAAATGGA TTA1TTGCTC ATGATTTTCT  
 3901 CTCTGCTGTT TGTGGCTTGC CAAGGAGCTC CAGAAACAGG TAGGCACGCT CGTTGACTTG  
 3961 TAAGTCTCGG AATTACAGAT TAGTGTGTTT TTATCCACCT TCATGCTTTT CTTGCTCTA  
 4021 TTTTTCCTCCG TTTCTTTTAT GACTGCAGCT TAGAGAGCAA GTGCTGAGA ATTATGCTG  
 4081 AAACGTACTT TAAGTCTCTT AGTGTAAAT GTAAATTTCC TCTACTGAAT ACAATTAAGT  
 4141 GCAATTGACT ATACATGAC ATTAAATATA CTATCGTTT TATTAATTAT ATTCCATTAT  
 4201 GTGTTTCCCT GCGTTTAAA AAATGAGAAG AGTATGGACA TATACAATT AGTCAAATGT  
 4261 ATGTTTGTAA TATATGTGTT TATACAGGTA CACAGGCCAT ATAGGAACCT AAATCTTATT  
 4321 TAAACACTAT TTTAATAGTG TGTAAACGTG TAAATATTTT AAGCATTTCA GCTTGAAGCC  
 4381 AAGGAATTGT ATCCAGTCGT TCAGGCAATG TATGTTGAGT AAAATCACC GCAGAGCAAA  
 4441 AGTCTGTTGA CTAACCTACC CTTCCCCCGC CCCCCCACC CCCCCCGCAG GCGGTTCTG  
 4501 GTGAAGCAG ATGTTTCTT TAAATTTTGT CATCATTTGAC TTTAGGTTTC TTTTGGCAGG  
 4561 TTTTGGCAC CCAAAACAGT GTGAGCTCTC TTTTCAAGCTT TATTCACTG TGCTGGGAGG  
 4621 GGAGCTAGGA TAATTTCTGG CTGCCGAAGG ATTTAGGCAG TCGGTGTGCA TCTGCCCGGG  
 4681 TCCCCCCCCG TTTTAGGGTC AGTGCACTTT TTTTGTCTTT TCGTGACCC GACTAAAGAG  
 4741 AAAGGATGTC AAGGAATGA AAATCCTGGA ATGTGCTGA TCATTTGAAA TGTACAAAT  
 4801 TGGGCAGATA AGCTGCATGG CTAAATTGTT AGGAGGAAGA GGCAAGGCAG TAGTGAGAA  
 4861 GGGGAGGCA GTGATCCCA CACAAGCCTG ATGCCAGGG ATTGGGAATT CAAAATCCCC  
 4921 CCAGCCTACC TTCAGTCCC TGACCTGCTT CTCAGCCCCA CCTTAGGTCA CTGGTTCTA  
 4981 TGGAGTTACC CTACTGAATT GAATATTGAA TAGTTAATT CTCCTCCAA TCATTTTCCC  
 5041 CACCTAATTT TGAAGATAT ACATCATCTG GGGTACCCTG TGCCCTACAC AGCATGTGAA  
 5101 GTGATGGGT ACCCCCTAAA GAGAGGCTCA TCCCTGAATG GGAAGTGCC CCAAGCTAG  
 5161 GAATTAACGT GATTTCTTGT CTTTGTGAGT GTGCCAATGT TAAGTAAGCT TCAGTGATA  
 5221 GTGCTGTCTT ACCAAGTTCC TTGTAGAAGC CAGCCGATTT TTCAACAGGC AGCATTTCCAC  
 5281 AGCATTTCCC TGAGCCTGCT TCAAGAGGGG TGGGGGAAGT CCCTTTTACG GTGTTATCT  
 5341 CCTCTGCATT TGTGTAATCT CCCTGAAGGT GGATTAAGCA AGGCATGAG GGGGAGGCAA

Figure 1-4

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5401 AAGGTGAAC TATGTTAAG AGGAAAAA ATAAGAGCC CTTTTTCTG TGTTCCTGC  
 5461 TGATGGCAGG CTGTGTGCTT CATCTGCTT TATCTGCTT GCTAGCTCTG ACTCTACTGT  
 5521 GATCCAGCAT GTCTCTCGGC GTTTGAGAG ACATCCCCCA CTGACCTGCT CTTCTCTCC  
 5581 CCAGCAGTCT TAGCGCTGA GCTCAGCCG GTGGTGAGA ACGCGGGGA GAAACCCACT  
 5641 CCCAGTCCAC CCTGGCGCT CCGCCGCTC AAGCGCTGCT CCTGCTGCT CCTGATGGAT  
 5701 AAAGAGTGTG TCTACTCTG CCACCTGAC ATCATTTGGG TCAACACTCC CGAGTAAATC  
 5761 TCTAGAGGGC ATTGTAACCC TATTCATCA TTAGCGCTGG CTCACCTGGA GCCCAGTTT  
 5821 AGAGTTTCTT TTCTAGGGAC TCTGAAGTA GTCTTCTTAA CACCATCCAA GTGCCCTAGT  
 5881 GGGGACAGTT TCCCTCTATT CTTGAAAAA ACGACAGCTT CGTCTTTAGC AACCAAGGGG  
 5941 AGGCTCTCT GAGCCCCGT AGCTCAGCT ACTCATGATG GGACAAGCAG GAGGCCACTG  
 6001 CACGTTTCAA ATGAGGAAC TTTAGTGAGA GGGCTCAGG GGGACACTCT CACAGTGGA  
 6061 TCTGATGGG TTTGGGAAT AATTGCCGAG GTGAGATGTG GGTAGTGCA ACCGTGCTT  
 6121 CTCATGGAG GGTGAGACT GAGAGCAGA AGTGATGATA TAGAGGTTA GAATCACTTA  
 6181 ATTTAGTTA CAGAAAAAC TAGGCTCAA GTGTGAAGC CATTTGTGCA GGAGTGAAT  
 6241 TGTAGCAGAG CTGAACCTGG AGCCCCGATT TCCCTTCTG CTATATTTT CCTTTAGAAA  
 6301 TGCCCATTT AGAAGTGA A TAGAATACT GTCCATAGGC TTCTCTTTCA CCTACAGAGA  
 6361 AGAAAAAGCAG ATTTCTCTCT TCTGCCCTGG ACACCTAGTT ATCATCTGTC GGAAGCAGTC  
 6421 ATAAACAAGC ACACATTTAC TATGCAATACA ATGTACCGTT ATGACAAAGG AGGACCAAAA  
 6481 TCCAACAAT ATCAAAACC ACCAAAAACC ACAAGAGCC TAATTAATTAC TAAGTGATA  
 6541 CTTCCAAGG GAGGACTTTA TTTCTTAGAT GAGAAAGAA ATGACACAT TGGAAATTAT  
 6601 TGAGAGCCC TCTGGCTATG AGTCTTCCA CAACCATATG GTACCACCGA CTGGCAGGAG  
 6661 AAATGTGTA ACATGTGCTT CTTCTCCCCA ACCACTGGG CCGGTGGGT GACGGTGGCA  
 6721 CTTTTCAGC TATCTCTGCT GGTTCGAGTT GAAAAATAGT TTTAAAAATC CTGTGAGTCA  
 6781 TGGTTTGA TTGAACCTC TTCCCACTGT GTACCACCAA ATAGTTAAT AAATAGACCA  
 6841 TTAGAAAAAG AAGAAATAT AAAGCAGATG CCAAGCAGAG ATGTCTTAAT TTTTGACAAA  
 6901 AAAGCAATGT TGTGTGTCT AAGAGAAAC TGAACCTTGT GAAGAGTTGA AATGGAATTC  
 6961 CACTGAATTA GAAAAACTTG TTTTCTCTG CCTGATACA TACAGTCAGG GCCATTTGATG  
 7021 CACAGGTGT CTTGGCTGTT GTTACACTTT ACCCTCTGAA ATGATGCTCC CAAGTCTAT  
 7081 GTGATGAGCT CCTGTGTGC CCAGTGAAT AGGTGTGTC ATGTGTCAAT TTAAGACTA  
 7141 TTAATTACAC TAATATAGTT TCTTCTCTC TTTGATTAAT AGGACGTTG TTCCGTATGG

Figure 1-5

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7201 ACTTGAAGC CCTAGGTCCA AGAGAGCCTT GGAGAAATTTA CTTCACCAA AGGCAACAGA  
7261 CCGTAGAAT AGATGCCAAT GTGCTAGCCA AAAAGACAAG AAGTGCTGA ATTTTGCCA  
7321 AGCAGGAAAA GAACTCAGGT GAGCAGAAAC ACCTTTGCTT TTCAATCAGT TTAACAGCCT  
7381 CCTGAACCTCC TTCCATATCAT GGTACTGCCCT TCCTGTTTGA GAGAGACTAA CAGAGACATT  
7441 GAAAGTCAGG GTAAAGCTGA ATATACATTT GCTGAATGT TTTTCCTTGT GTATTTTAAC  
7501 AGGCGTGAAG ACATTATGGA GAAAGACTGG AATAATCATA AGAAAGGAAA AGACTGTCC  
7561 AAGCTTGGGA AAAAGTGTAT TTATCAGCAG TTAGTGAGAG GAAGAAAAAT CAGAGAAAGT  
7621 TCAGAGGAAC ACCTAAGACA AACCAAGTAA GAGGGAAGGA AGAAAAATTA GGTAGAGGT  
7681 TCACAAGAAC AACTAGCCCC AGTCAGTGAT GCCAGCAGCC TGTTCCTCCA GCCCTTCTTA  
7741 CCGGGCAGG TGAAGAAGCTT AGAAAAAGT AGCAGAGAG ATCTATGCAT CCTATAGATT  
7801 AAAAGAGACA AAAGAATCCC TCTTAATAT TTCCATGAAG CTCTGGAATG CAACCGATG  
7861 TCCTCTGTAC CTTTAGCACA TACCATTTC AAGCCTTGG CCTCTCTGAG TCAATGTATT  
7921 TCCAGAGATG CTTTGTCTAT TGGCTTATAT ACAGCCTTGG CCTCTCTGAG TCAATGTATT  
7981 TACCACCTTC CCTGAGAAAT CGAAATTCAT TTTGGGAGC GGACATTTAG AAAAAGAAATC  
8041 AAGTGTCAAT GGATAATCAA ATTCCTCAAT AAGTTCAGT TATTCAGATG GCCAAAGGAA  
8101 AAATAAAGTC ATTAGATAGG GTTGGTAGAA TTTAGAACAT GCTGTTTTTC AGGTTATG  
8161 TCTTTTTTTT TTTTTTTTTT TTTTCTGCT CAGTCATGTG CTGGACAGA GAAGGATCT  
8221 TCATTTCCAAA AAGCTCTCTC TTTTCTGCT CAGTCATGTG CTGGACAGA GAAGGATCT  
8281 GGATTAGGCA ACATCATAGA GTTGCCTGA GCTGCTCTTT GGTGATAACC CTTCCAAATC  
8341 CTAAACTTTT TGGAATTCAC AAGCTCAAG GAGGAACCT ACTCTCTGAT CTACACATG  
8401 TTCTGCATTT TTCTATCATG GTCTATGGA ACTTCTCTTA GAAATCCAGT GGCAAGAAGT  
8461 TCTATGATTA AAGTGTCTG AGCTCAGGCC AGGCACTCAT GAACCTACTTC TGAGTTGTTT  
8521 ACTACTGATT TGTGGGGCAG CCTCAGCTAT CGGTTCCTC ACACCTGCTT ATGAGAGTAT  
8581 CCATATTTAT GGTCCAGGC AGTAATGCTC CCCACGAGAT CAGTTTCTGA ACTAACCTGG  
8641 AATTTTTTAT GGGTTTTTAT TATGCCAAT ATTAAATCAA CATTACAGTT CTTCCTCTG  
8701 TATTTCTCT GTAAACATTT AGGCTGCAA AAAAAAATAA TCTTTTAA AAATAATGCC  
8761 ATAAAGTATT TGCTCTGGCC CTACTGTATG CTCTTTTTT TTTTCTCTCT TTTCAACTAA  
8821 GTCAACCTCA ATTTATTAAG ATGGCATAAA CTATTCAAAA CCTATGCTGA GTTCCTCAAG  
8881 GCAGGCTGC ATAGTATGA AGGTGGGAT GGGCTACGG AAGAAACAG AACCACTCTA  
8941 GTTTATTTAA AACCTGTATT TACTGCCAC TTCCCTTAG ACTGACCAT ATGACCCCTT

Figure 1-6

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9001 GCTCCCCATT CTAAGCATAG GGGCAGGCTT TATTTTACA ATGGTAATAG ATGATATCAC  
 9061 TTGAGGTTTAT ATCAAGAGT TGCGCGGGGT GGTGAAGTT CACAACCAGA TTCAGGTTT  
 9121 GTTGTGCCA GATCTAAT TTACATGTTT CTTTGGCCA AGGGTGAATT TTTTAAATA  
 9181 ACATTTGTTT TCTCTATCT TGCTTATTA GGTGGAGAC CATGAGAAAC AGCGTCAAT  
 9241 CATCTTTTCA TGATCCCAAG CTGAAGGCA AGCCCTCCAG AGAGCGTTAT GTGACCCACA  
 9301 ACCGAGCACA TTGGTGACAG ACCTTGGGG CCTGTCTGAA GCCATAGCCT CCACGGAGAG  
 9361 CCCTGTGGCC GACTCTGCAC TCTCCACCTT GGTGGGATC AGAGCAGGAG CATCCTCTGC  
 9421 TGGTTCCTGA CTGGCAAGG ACCAGCGTCC TCGTTCAAAA CATTCACAGA AAGGTTAAG  
 9481 AGTTCCCCCA ACCATCTTCA CTGGCTTCCA TCAGTGGTAA CTGCTTGGT CTCTCTTTC  
 9541 ATCTGGGGAT GACAAATGAC CTCTCAGCAG AAACACACAG TCACATTCCA ATTCGGGTGG  
 9601 CATCCTCCGG AGAGAGAGAG AGGAAGAGA TTCCACACAG GGGTGGAGTT TCTGACGAAAG  
 9661 GTCCTAAGGG AGTGTTGTG TCTGACTCAG GCGCCTGGCA CATTTCAGGG AGAACTCCA  
 9721 AAGTCCACAC AAAGATTTTC TAAGGAATGC ACAATTGAA AACACACTCA AAAGACAAC  
 9781 ATGCAAGTAA AGAAAAAAA AAGAAGACT TTTGTTTAAA TTTGTAAAT GCACAACTGA  
 9841 ATGAACCTGT TACTACATA AATCAGAGTA TGTTCATGA ATATGAGTCT ACCTCACCTA  
 9901 TATTGCACCTC TGGCAGAAGT ATTTCCACA TTTAATTATT GCCTCCCAA ACTCTTCCA  
 9961 CCCCTGCTGC CCTTCTCTCC ATCCCCATA CTAAATCCTA GCCTCGTAGA AGTCTGGTCT  
 10021 AATGTGTCAG CAGTAGATAT AATATTTTCA TGGTAATCTA CTAGCTCTGA TCCATPAAGAA  
 10081 AAAAAAGATC ATTAATCAG GAGATTCCCT GTCCTTGATT TTTGGAGACA CAATGGTATA  
 10141 GGGTTGTTTA TGAATATAT TGAAGAATA GTGTTGTTA CGCTTTAAG CAGTAAATTT  
 10201 ATTTTCCTT ATATTAACCG CTAAATGAAG AGGTGGATT GAATTTTGAT GTACTTATTT  
 10261 TTTTATAGAT ATTTATATTC AAACAATTTA TTCCCTTATAT TTACCATGTT AAATATCTGT  
 10321 TTGGGCAAGC CATATTGGTC TATGTATTTT TAAATATATGT ATTTCTAAT GAAATTGAGA  
 10381 ACATGCTTGG TTTTGCCGTG CAAGGTAATG ACTTTAGAAA ATAATATTTT TTTTCCCTTAC  
 10441 TGTACTGATT TGGAAATCAT ACTGAAATTT GTAAGGAGTG GCCCAACGTG ATTAAGTACC  
 10501 ATAAAGCAA ATAATGCTT AAAGACGCTT TCATAGAAAA GTGACAAATTA GAAGGATATT  
 10561 ACGGCTTAAG CTAATTATAT AAAGAATTTT ATCTGTATCT TAAATGTGA TTTTATACCTG  
 10621 CATTGAGGTA AAAACACAAA ACAAAAAAGC AGCTTTAACA CCTCTGTCTT CTCTTGGGTA  
 10681 GCAGCCTCCT GCTTCTCCTT CACCTGAAAA ATTCCTCAGG GACTTCATCC ATTAACCTGG  
 10741 CTCAGGCTAT TGGCAGGATT CACAGTTTAA GCTGATGGTG TGGTGAGAGA TGCTTATACC

Figure 1-7

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10801 ATATTAATGG ACTGAAGGAA GTAATGGCAA GACAACCCCC CAAACATAC CTAATTATAC  
10861 AAAGTTATAT ACCAAGTTG CTTTAGAATA ATGGCCCTGCT CAGAGCAAGT AGAGTTTCC  
10921 AATGGCTTTT TATTTCTCA CATTAGGAT GTTGTTCCTT AAGAACATTT GAGTACCATT  
10981 GCTCTCTCGT GATAGCCCTAG GACTGCCCTG TGCCCATGGA GGTAGAGACA CCAGTACTG  
11041 ATCTAGGTC CTCTGCCACA AAGCAACCACT TCCTCTCCAC TTTGCCCTGG CTGGCCCTGT  
11101 CAGCTCACTG GAGAGCACAG TATTGCAATT GCAGTATTGC AAATGGTCAC TACTAATGA  
11161 ATCTCTAAG AGCTTGATTA GCCCTCGAGA ATCTTCCTTG CCTTCTCTA ATAGTGTCTG  
11221 AAGGAATCC TGGCATTTAA CAAATATTAG CATGTAGTGA TCACGTGCTT CCTAACAGTG  
11281 ACACATCAGA AGGATTTCAA ATAACAGTCT TCAGGCATGC GTAATCAATG TCCTGTGCAG  
11341 AGCTCCGTC CTCATTGATC CTCATTTTTC TCTTTAAGGC ACAGTCCAAT GTCTTTGGGG  
11401 AATGTTTAT AAAGCTTACT TTATCCATTA ACTGTTTCTC AGTGCCTGAC TCTGAAGAAA  
11461 ATTTGAGAT TTTGCCCATG TTGACAAAGT GCTGGTCTG AACTTGCCA GTATTTAATC  
11521 TTGAGCAAC GATTCAATTT CCTCTATCG TGAGTTTCT CATCTATGAA ACAAGGAGT  
11581 TGAGGGAGT TTCTTTCATA CCTCTGAGAA AGAGTTTGAG ATTACATAAA GAAGTTGAAG  
11641 TGGCATGAAA AAAAATAAAG ATCTGAGCTT AGAAGACATG GATCTAATAC ATTTAAGAGG  
11701 AAGTCAGAT CAGAGAAGCC ACTGAACAAA ACAGTCCAAA CGGAGCATAG TAAGTCAGAT  
11761 TGATGAGTTT TGGTTGGGT TTTTCATCAGT CAAACCCCTTG AGCCCCCTT TCCCATGCTT  
11821 CCTGCTTCAG TATCCAGTAG GAAAAATGAA AGGATGATG TAGACACTCT AGGCAATGAG  
11881 GATTTGCAGT AAATAAGTTG GGAGACTCAC AGAAATTA TATTTTCAA ACATGAAGAC  
11941 GAAACATCA ATTATATTAC AGTCCACATC AGCTGAGAG GTAACCTGAT GGGATGATCT  
12001 GTCACATTTT TTTGCTCTGT TCCAGTAATA GCATGGTTTC TGGAAACCCA CTTAGGACAG  
12061 CTTCTCTCTT TTACACTGAT AGCCCAAGCA AGCTTTGATC TCAGAACTCC AGAAACCAAG  
12121 GAACCTAGG TGGATGTGG TAACTTTTGC CAGGGCAGAG GGAACACCTA CTAATAGGTA  
12181 CTTCAATTTG ACCACCAAG AGTTGCATCT TTTTGTATGG ATCCACTGGC TTTGATACTG  
12241 CCTGTACTCC CCCAAAACAC AGCTTGGGTA TTGGAATTAAT CTAGAGCTCC CTCAGAGAGAA  
12301 CTCTTGCTGA CATTAGAAAA GAGCAACATTT TTGTCTTTC AGGTGAAAAAT CCAAGGCCAA  
12361 AAAGGAGGTG ACTCACTTAA GATCACAGAA GGAGCTGTAG CATCTCTGGA GCCTGAACAC  
12421 TTTAAGTTAAG CACGACTATT TCACGCAGAG GGCATGAATT C

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/02584

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/11 A61K31/70 A61M15/00 A61M11/00 //B65D83/14

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07H A61K A61M C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	<p>SAKAI S ET AL: "Pulmonary hypertension caused by congestive heart failure is ameliorated by long-term application of an endothelin receptor antagonist: Increased expression of endothelin -1 messenger ribonucleic acid and endothelin -1-like immunoreactivity in the lung in congestive heart failure in rats."</p> <p>JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, (1996 NOV 15) 28 (6) 1580-8., XP002092833</p> <p>see the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1,3-11, 16

☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 February 1999

Date of mailing of the international search report

24/02/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

Andres, S

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International Application No  
PCT/GB 98/02584

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	WO 96 40162 A (UNIV EAST CAROLINA ;NYCE JONATHAN W (US); METZGER W JAMES (US)) 19 December 1996 see page 7, line 2 see page 8, line 23 - page 10, line 11 see page 11, line 17 - line 27 see page 38, line 20 - page 39, line 35 see claims ---	1,3-11, 16
X	GB 2 283 179 A (HIGENBOTTAM TIMOTHY WILLIAM) 3 May 1995 see the whole document ---	17-21
X	WO 94 27664 A (KEANEY NIAL) L 8 December 1994 see page 5, line 8 - page 7, line 18 see claims ---	17-21
A	BUTT A.Y. ET AL: "Pathophysiological basis of the treatment of pulmonary hypertension." EUROPEAN RESPIRATORY REVIEW, (1995) 5/29 (248-251)., XP002092834 see page 249, right-hand column, paragraph 2 - page 250, paragraph 1 ---	1
A	STELZNER, T. ET AL.: "Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension" AMER. J. PHYSIOL., vol. 262, May 1992, pages L614-L620, XP002092835 see the whole document ---	1
P,X	GB 2 320 900 A (UNIV SHEFFIELD) 8 July 1998 see abstract see page 3, line 18 - page 5, line 5 see claims -----	17-21

# INTERNATIONAL SEARCH REPORT

international application No

PCT/GB 98/ 02584

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 1-2, 16 and 22  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-15,22  
Antisense oligonucleotides against endothelin-1 for the treatment of pulmonary hypertension.
2. Claims: 16  
A method of determining the efficiency of a therapeutic composition in the treatment of pulmonary hypertension.
3. Claims: 17-21  
An inhaler.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-15,22

Antisense oligonucleotides against endothelin-1 for the treatment of pulmonary hypertension.

2. Claim : 16

A method for determining the efficacy of a therapeutic composition in the treatment of pulmonary hypertension.

3. Claims: 17-21

An inhaler.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/02584

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9640162	A	19-12-1996	AU 6095996 A CA 2223776 A CN 1192686 A EP 0831848 A	30-12-1996 19-12-1996 09-09-1998 01-04-1998
GB 2283179	A	03-05-1995	AU 7819994 A DE 69412502 D EP 0723466 A ES 2123833 T WO 9510315 A US 5839433 A	04-05-1995 17-09-1998 31-07-1996 16-01-1999 20-04-1995 24-11-1998
WO 9427664	A	08-12-1994	GB 2279014 A US 5713349 A	21-12-1994 03-02-1998
GB 2320900	A	08-07-1998	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/GB98/02584		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 2 September 1998 (02.09.98)		<b>Published</b> <i>With international search report.</i> <i>With amended claims.</i>	
(30) Priority Data: 9718487.3      2 September 1997 (02.09.97)      GB		<b>Date of publication of the amended claims:</b> 15 April 1999 (15.04.99)	
(71) Applicant (for all designated States except US): UNIVERSITY OF SHEFFIELD [GB/GB]; Western Bank, Sheffield S10 2TN (GB).			
(72) Inventors; and (75) Inventors/Applicants (for US only): HIGENBOTTAM, Tim- othy [GB/GB]; University of Sheffield, Section of Res- piratory Medicine, floor F, Beech Hill Road, Sheffield S10 2RX (GB). McCORMACK, Keith [GB/GB]; Univer- sity of Sheffield, Section of Respiratory Medicine, floor F, Beech Hill Road, Sheffield S10 2RX (GB). SMITH, Adrian [GB/GB]; University of Sheffield, Section of Respiratory Medicine, floor F, Beech Hill Road, Sheffield S10 2RX (GB).			
(74) Agent: MARKGRAAF PATENTS LIMITED; The Crescent, 54 Blossom Street, York YO24 1AP (GB).			
(54) Title: ANTISENSE TREATMENT OF PULMONARY HYPERTENSION			
(57) Abstract  The invention herein described relates to a method to treat pulmonary hypertension by antisense therapy using ET-1 derived antisense molecules delivered to the lungs as a pulse/spike in an inhaler.			

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## AMENDED CLAIMS

[received by the International Bureau on 3 March 1999 (03.03.99);  
original claims 12 and 13 amended; remaining claims unchanged (4 pages)]

8. A therapeutic composition according to Claims 3-7 wherein said oligonucleotide has a G + C content of between 50% and 60%.
- 5 9. A therapeutic composition according to Claims 3-8 wherein said oligonucleotide does not contain a consecutive run of more than four bases of any single kind.
- 10 10. A therapeutic composition according to Claims 3-9 wherein said oligonucleotide has a predicted melting temperature of at least 55° C.
11. A therapeutic composition according to Claims 3-10 wherein said oligonucleotide has a predicted melting temperature of between 55° - 80°C.
- 15 12. A therapeutic composition according to Claims 3- 11 wherein said antisense ET-1 molecule is of human origin and comprises at least one from:
- i) CAG CCC AAG TGC CCT TTA AC (SEQ ID NO:2)
  - ii) CTC AAA GCG ATC CTT CAG CC (SEQ ID NO:3)
  - 20 iii) AGC TCA GCG CCT AAG ACT GC (SEQ ID NO:4)
  - iv) TGG CAG AAG TAG ACA CAC TC (SEQ ID NO:5)
  - v) CCA AAT GAT GTC CAG GTG GC (SEQ ID NO:6)
  - vi) TGG TCT CCG ACC TGG TTT GT (SEQ ID NO:7)
  - vii) ATG TGC TCG GTT GTG GGT CA (SEQ ID NO:8)
- 25 or an oligonucleotide sequence homologous therewith.
- 13 A therapeutic composition according to Claims 3-12 wherein said

antisense ET-1 oligonucleotide is of rat origin and comprises at least one from;

- i) ACA GCA GAG AGA AGA TCA CG (SEQ ID NO: 9)
- ii) TGC ACT TCC TTC TCA GCT CG (SEQ ID NO:10)
- 5 iii) GGA TCG CTT AGA CCT AGA AG (SEQ ID NO:11)
- iv) CTT GAT GCT GTT GCT GAT GG (SEQ ID NO:12)
- v) AGT CAA TGT GCT CGG TTG TG (SEQ ID NO:13)
- vi) ACT GTG TCT CTG CTC TCC GA (SEQ ID NO:14)
- vii) CAC CAG CTG CTG ATA GAT AC (SEQ ID NO:15)
- 10 viii) CTG TAG TCA ATG TGC TCG GT (SEQ ID NO:16)
- ix) GGC TCT GTA GTC AAT GTG CT (SEQ ID NO:17)
- x) CCT CTG CCA GTC TGA ACA AG (SEQ ID NO:18)
- xi) CCA GAC AGC AAG AAG AGG CA (SEQ ID NO:19)
- xii) GGA ATG GCA CTG TGT CTC TG (SEQ ID NO:20)

15 or an oligonucleotide sequence homologous therewith.

14. A therapeutic composition according to Claims 3- 11 wherein said non- coding strand of the ET-1 gene is adapted to hybridise to the transcriptional promoter region of the ET-1 gene to inhibit transcription of  
20 said gene.

15. A therapeutic composition according to Claims 3- 11 wherein said non-coding strand of the ET-1 gene is adapted to hybridise to splice junctions between exons and introns of prepromessenger RNA encoding the  
25 ET-1 protein.

16. A method for detemining the efficacy of a therapeutic composition in



the treatment of pulmonary hypertension comprising administering a composition according to Claims 3-15 to a selected human or animal and observing the effects of said composition on pulmonary hypertension.

- 5 17. An inhaler comprising means for administering the therapeutic composition according to Claims 3-15, wherein said therapeutic composition is administered via a pulse or spike during use of the inhaler.
18. An inhaler according to Claim 17 wherein the inhaler is disposable.
- 10 19. An inhaler according to Claim 17 wherein the inhaler is adapted to receive a cartridge containing antisense molecules according to Claims 3- 15.
20. An inhaler according to Claims 17 -19 wherein said inhaler comprises a removable cartridge wherein said therapeutic composition is stored prior to use.
- 15 21. An inhaler according to Claims 17-20 wherein said inhaler comprises a rechargeable cartridge in which said therapeutic composition is stored prior to use.
- 20 22. A method for administering a therapeutic composition according to Claims 3-15 comprising;
- 25 i) adding or providing said therapeutic composition in a suitable dilutant, carrier or excipient and in dosage form to, or in a reservoir of, an inhaler;
- ii) aerosolising said composition by suitable means;

- iii) detecting the air pressure within the inhaler by suitable sensing device to determine a patient's respiratory cycle; and
- iv) delivering a prescribed dose of the therapeutic composition to the patient's lungs during a selected inhalation phase of the patient's respiratory cycle.

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22. A kit for treating pulmonary hypertension comprising a therapeutic composition according to Claims 3-15, an inhaler according to Claims 17-21, an air pressure sensing device, and a valve containing pressurised cylinder means to regulate the dosage of said composition.

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